REMARKS

Claim 1 has been amended to recite that the glucose dehydrogenase expression is enhanced. Claims 1-6 and 8-9 are now pending in this application. Support for the amendments is found in the existing claims and the specification as discussed below. Accordingly, the amendments do not constitute the addition of new matter. Applicant respectfully requests the entry of the amendments and reconsideration of the application in view of the amendments and the following remarks.

Rejection under 35 U.S.C. § 103(a)

Claims 1-6 and 8-9 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Sode, et al. (WO 2002/36779), in view of Herbaud, et al. (BBA 2000, 1481 (1): 18) as evidenced by Arslan, et al. (BBRC, 1998, 251: 744).

Applicants have amended claim 1 responsive to the comments of the Examiner in the Advisory Action of March 28, 2008 that the enhancement of GDH activity discussed in the previous response is not a claim element. Support for the amendment is found in the present specification at page 20, 2nd full paragraph and pages 20-21, bridging paragraph, for example. Reconsideration is requested in view of the amendment and the remarks below.

Applicants' claimed invention is directed to co-expression of the α and β subunit of glucose dehydrogenase (GDH) of *Burkhorderia cepacia* (and optionally the γ subunit, claim 3) in combination with genes of a ccm operon. The GDH activity obtained with the claimed invention was unexpectedly high which could not have been predicted based upon the combination of references.

The Final Office Action states that

> ...all of the claimed elements were known in the prior art and one skilled in the art could have combined the element as claimed by known methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. Each of the elements (α- and β- subunit of GDH; ccm Operon; and [sic]) are taught by Sode in view of Herbaud et al. and as evidenced by Arslan et al. It would be therefore predictably obvious to use a combination of these three elements in a recombinant Escherichia bacterium. (Office Action, page 5, lines 7-14).

However, neither Arslan, et al. nor Herbaud, et al. teach or suggest that expression of a ccm system in E. coli has any effect on a glucose dehydrogenase (or other enzyme) activity. Both Herbaud, et al. and Arslan, et al. merely indicate that, at least in some cases, expression of the ccm genes facilitates production of mature cytochrome c.

In contrast, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). This increase in GDH expression and activity could not have been predicted based upon the disclosure of Herbaud, et al. and Arslan, et al. on stimulation of cytochrome C levels, especially as the stimulation in maturation of cytochrome c was not observed in all cases as discussed above. Claim 1 has been amended to recite the enhanced GDH expression as a claim element.

Additionally, Applicants argue that unexpected results were obtained with the claimed combination compared to the prior art. As stated above, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). By co-expressing the enzyme complex including the γ-subunit, the α-subunit and the β-subunit with the ccm genes, the GDH activity in *Escherichia* bacterium increased to a level that was unexpected (present specification, page 20, second full paragraph and pages 20-21, bridging paragraph) compared to expressing the enzyme complex only and the wild type strain. While the activity of the recombinant *E. coli* which also included the genes for the ccm operon (JM109/pTRC99Aγαβ, pBBJMccm) was 32 U/mL, the two controls had activities of only 0.3 (JM109/pTRC99Aγαβ) and 1.4 (*Burkhorderia cepacia* KS1). Such high expression levels could not have been predicted from the cited references.

The Final Office Action states that the references "also suggest unexpectedly levels of GDH" (Final Office Action, page 5, 6th line from bottom). However, as discussed above, Herbaud, et al. and Arslan, et al. teach effects on cytochrome C levels, not GDH activity. Furthermore, Herbaud, et al. report that the highest amounts of cytochrome c produced were on the order of 300 μg/L of culture when 0.1 mM δ-aminolevulinic acid was included along with the ccm system under aerobic conditions (page 22, col. 1, first partial paragraph) which is about the same as what could be produced in *D. vulgaris* (page 22, col. 1, first partial paragraph).

Accordingly, Herbaud, et al. do not show levels of cytochrome C production that are unexpected and do not show any effect on activity of an enzyme such as GDH.

Sode, et al. teach the β subunit of GDH but does not suggest a method combining GDH with ccm.

Furthermore, Applicants continue to argue that it was not predictable at the time of the claimed invention that the presence of the ccm operon would result in an increased level of cytochrome C as indicated by Arslan, et al. who teach that inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production of cytochrome c (see page 747, col. 1, last paragraph). They conclude that low levels of ccm gene products must be present already and that addition of ccm genes (pEC86) therefore produced no stimulation.

Although Arslan, et al. teach instances where cytochrome maturation genes (ccm) increased production of both endogenous and foreign c-type cytochromes (see Abstract), in at least one instance stimulation of cytochrome c production by expression of ccm genes was not observed as inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production (see page 747, col. 1, last paragraph).

Accordingly, the claimed microorganism and method provide improvement in both GDH expression as well as enhanced expression of ccm in the presence of the ccm system which could not have been predicted based upon the combination of cited references.

In view of Applicants' amendments and arguments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

No Disclaimers or Disavowals

Although the present communication may include characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

In view of Applicants' amendments to the claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: May 30 2008

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